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金铁锁根中的环肽成分*

丁中涛[1,2]，汪有初[1]，周　俊[1]，谭宁华[1]，吴厚铭[3]**
（1 中国科学院昆明植物研究所植物化学开放实验室，云南　昆明 650204；2 云南大学化学系，
云南　昆明 650091；3 中国科学院上海有机化学研究所生命有机国家重点实验室，上海 200032）

摘要：从云南民间重要的药用植物金铁锁（*Psammosilene tunicoides* W. C. Wu et C. Y. Wu）**的根中分离得到** 2 **个新的天然环二肽以及** 2 **个新的环八肽：金铁锁环肽** A **和** B（psammosilenins A and B）. **它们的结构经光谱方法鉴定为** cyclo（– Ala – Ala –），cyclo（– Val – Ala –），cyclo（– Pro_1 – Phe_1 – Pro_2 – Phe_2 – Phe_3 – Ala – Pro_3 – Leu）**和** cyclo（– Pro_1 – Gly – Phe_1 – Val – Pro_2 – Phe_2 – Thr – Ile –）.

关键词：金铁锁；石竹科；环肽

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Cyclic Peptides from the Roots of *Psammosilene tunicoides* *

DING Zhong – Tao[1,2], WANG You – Chu[1], ZHOU Jun[1**],
TAN Ning – Hua[1], WU Hou – Ming[3]
（1 *Laboratory of Phytochemisty*, *Kunming Institute of Botany*, *Chinese Academy of Sciences*, Kunming 650204, China;
2 *Department of Chemistry*, *Yunnan University*, Kunming 650091, China;
3 *State Key Laboratory of Bio – Organic Chemistry and Natural Products*, *Shanghai Institute of Organic Chemistry*, *Academia Sinica*, Shanghai 200032, China）

Abstract: From *Psammosilene tunicoides* W. C. Wu et C. Y. Wu, two new natural cyclic dipeptides and two new cyclic octapeptides (named psammosilenins A and B) were isolated. Their structures were determined as cyclo（– Ala – Ala –）, cyclo（– Val – Ala –）, cyclo（– Pro_1 – Phe_1 – Pro_2 – Phe_2 – Phe_3 – Ala – Pro_3 – Leu –）and cyclo（– Pro1 – Gly – Phe_1 – Val – Pro_2 – Phe_2 – Thr – Ile –）by spectroscopic methods respectively.

Key words: *Psammosilene tunicoides*; Caryophyllaceae; Cyclic peptide

Psammosilene tunicoides W. C. Wu et C. Y. Wu, a monotype genus plant belonging to Caryophyllaceae, is a famous medicinal herb in Yunnan Province. It is used as anodyne and haemostatic (Wu, 1990). Some studies on its saponins have been reported (Pu*et al*, 1989). As one part of our investigation on the new cyclopeptides from the higher plants (Tan *et al*, 1993; Zou et al,

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** Author for correspondenceshould be addressed

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1993；Zhao *et al*，1995；Zhang *et al*，1997；Wang *et al*，1999)，a continuation of our investigation on the roots of this plant led to the isolation of two new natural cyclic dipeptides (**1**，**2**) and two new cyclic octapeptides (psammosilenins A and B) (**3**，**4**). This paper describes the isolation and structure elucidation of these compounds.

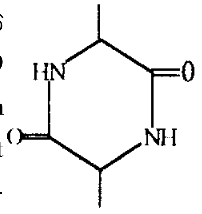
Results and Discussion

Compound 1 colorless needles，negative to ninhydrin reaction but positive after hydrolysis with 6 mol/L HCl. Its HREI – MS showed the molecular ion peak at m/z 142.0733，in agreement with the molecular formula $C_6H_{10}N_2O_2$ (calcd for $C_6H_{10}N_2O_2$ m/z 142.0742). IR spectrum exhibited intense NH and C = O absorptions at ν = 3300 and 1650 cm^{-1} respectively. The ^{13}C NMR spectrum showed the presence of an amide carbonyl at δ170.2，indicating that 1 was a symmetrical cyclic dipeptide.

DEPT spectrum showed one methine at δ51.2，one methyl at δ19.1 and one amide carbonyl at δ170.2. The ^{1}H NMR spectrum showed the presence of one amide proton at δ9.30 (br. s)，one methine at δ4.28 (q，J = 8.0Hz) and one methyl at δ1.63 (d，J = 8.0Hz). These results indicated this cyclic dipetide contained two alanine units，and its structure was elucidated as cyclo (– Ala – Ala –).

Compound 2 colorless needles，negative to ninhydrin reaction but positive after hydrolysis with 6 mol/L HCl. Its HREI – MS showed the molecular ion peak at m/z 170.1068，in agreement with the molecular formula $C_8H_{14}N_2O_2$ (calcd for $C_8H_{14}N_2O_2$ m/z 170.1055). IR spectrum exhibited intense NH and C = O absorptions at ν = 3300 and 1650 cm^{-1} respectively. The ^{13}C NMR spectrum showed the presence of two amide carbonyls at δ171.5 and 167.5. These facts indicated that 2 was a cyclic dipeptide.

Further analysis on the DEPT spectrum showed the presence of three methines at δ 60.9，51.2 and 32.2，three methyls at δ 20.9 19.1，and 17.1. The ^{1}H NMR spectrum showed the presence of two amide protons at δ9.25 (br. s) and 8.98 (br. s)，three methines at δ4.35 (m)，4.31 (m)，2.67 (m)，and three methyls at δ1.66 (d，J = 4.0Hz)，1.14 (d，J = 8.0Hz) and 1.07 (d，J = 7.2Hz). These facts led to the assignment of 2 as cyclo (– Val – Ala –).



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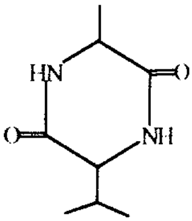

2

Psammosilenin A (3)：white powder，$[\alpha]_D^{24}$ – 108.1 (c 0.39，MeOH)，negative to ninhydrin reaction but positive after hydrolysis with 6 mol/L HCl. Its molecular formula was assigned as $C_{51}H_{64}N_8O_8$ by HR – FABMS [$(M + 1)^+$ at m/z 917.4859，calcd. m/z 917.4925]，indicating 24 degrees of unsaturation. The IR absorptions at 3290 cm^{-1} and 1640 cm^{-1} were attributed to amino and amide carbonyl groups respectively. The^{13}C NMR spectrum contained eight signals due to amide carbonyls at

δ174.1，173.5，173.1，172.8，171.7，171.5，171.5，170.5. The ^{1}H NMR spectrum exhibited five amide protons at δ 9.22，9.35，9.43，9.55，9.70. These facts indicated that 3 was a cyclopeptide.

In order to identify spin systems of different amino acid residues，2D – NMR techniques were used. By analyzing ^{1}H – ^{1}H COSY，HMQC，HMBC spectra，these amino acid residues were revealed to be one alanine，one leucine，three phenylalanine，three proline units. The molecular weight of these amino acid residues was identical with that observed in FABMS. Unambiguous assignment of ^{1}H and ^{13}C NMR signals（Table 1）was carried out by means of 2D – NMR techniques including ^{1}H – ^{1}H COSY，HMQC and HMBC.

HMBC spectrum provided the evidences for the linkage of the amino acid residues. It showed the connectivity of NH$_{Ala}$ to C = O$_{phe3}$，NH$_{Phe3}$ to C = O$_{Phe2}$，NH$_{Phe2}$ to C = O$_{Pro2}$，δ – H$_{pro2}$ to C = O$_{Phe1}$，NH$_{Phe1}$ to C = O$_{Pro1}$ and NH$_{Leu}$ to C = O$_{Pro3}$（Figure 1），which implied the presence of two peptide fragments of（ – Pro$_1$ – Phe$_1$ – Pro$_2$ – Phe$_2$ – Phe$_3$ – Ala – ）and（ – Pro$_3$ – Leu – ）. These two peptide fragments had to be linked in only one sequence. Consequently，the structure of 3 was determined to be cyclo（ – Pro$_1$ – Phe$_1$ – Pro$_2$ – Phe$_2$ – Phe$_3$ – Ala – Pro$_3$ – Leu – ）. The proposed structure was further confirmed by FABMS.

Table 1　^{1}H and ^{13}C NMR Data of Psammosilenin A（3）in pyridine – d$_5$（500MHz for ^{1}H NMR，125MHz for ^{13}C NMR）

	CO	Cα	Cβ	Cγ	Cδ	HN	Hα	Hβ	Hγ	Hδ
Leu	170.6	52.6	31.9	26.7	21.3 23.6	9.35（d） J = 8.5	5.02（m）	2.79（m）	2.07（m）	0.91（d） J = 6.5 1.0（d） J = 6.5
Ala	172.8	49.5	15.6			9.70 （br.s）	5.31（m）	1.31（d） J = 6.5		
Phe$_1$	171.7	55.9	37.9	140.3	126.5 – 130.4	9.43（d） J = 8.5	5.07（m）	3.79（m） 3.70（m）		6.95 – 7.46
Phe$_2$	171.5	54.6	38.3	136.9	126.5 – 130.4	9.55 （br.s）	5.24（m）	3.12（m） 3.26（m）		6.95 – 7.46
Phe$_3$	174.1	55.4	39.1	138.4	126.5 – 130.4	9.22（d） J = 6.5	5.33（m）	3.60（m） 3.28（m）		6.95 – 7.46
Pro$_1$	171.5	61.6	30.0	21.9	47.4		4.36（m）	1.23（m）	0.87（m）	3.54（m） 3.39（m）
Pro$_2$	173.5	59.7	29.6	25.7	47.0		4.89（m）	2.34（m） 2.07（m）	1.53（m） 1.95（m）	3.78（m）
Pro$_3$	173.1	61.0	30.7	22.8	47.5		4.74（m）	2.03（m）	1.73（m）	3.74（m）

Psammosilenin B（4）：white power，$[\alpha]^{24}_{D}$ – 73.6（c 023，MeOH），negative to ninhydrin reaction but positive after hydrolysis with 6 mol/L HCl. Its FABMS gave a $[M + 1]^{+}$ ion at m/z 859. The IR spectrum exhibited intense NH and C = O absorptions at 3300 cm^{-1} and 1650 cm^{-1} respectively. The ^{13}C NMR spectrum showed the signals of eight amide carbonyls between δ 169.1 and 173.3. The

^{1}H NMR spectrum showed six amide protons between δ 8.58 and 9.56. From these facts, 4 was deduced to be a cyclopeptide.

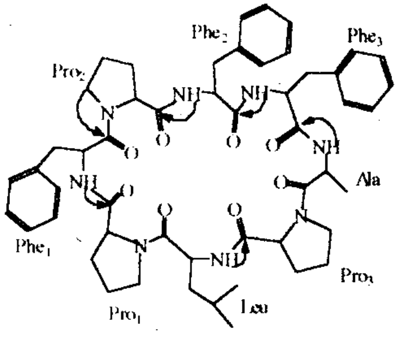

Fig. 1 Selected HMBC for Psammosilenin A (3)

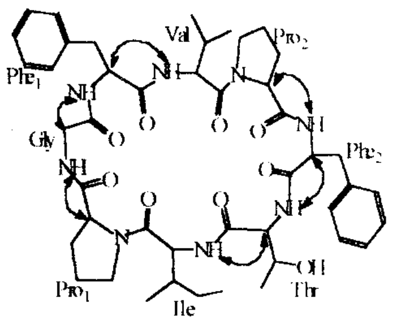

Fig. 2 Selected ROESY for Psammosilenin B (4)

Table 2 ^{1}H and ^{13}C NMR Data of Psammosilenin B (4) in pyridine - d_5 (600MHz for ^{1}H NMR, 150MHz for ^{13}C NMR)

	CO	Cα	Cβ	Cγ	Cδ	HN	Hα	Hβ	Hγ	Hδ
Gly	169.1	43.6				9.28 (br.s)	4.56(dd) J = 16.8, 9.0 3.36(d) J = 16.8			
Val	171.5^a	55.2^b	33.2	19.0 20.1		9.08 (br.s)	5.23(m)	2.56(m)	1.35(d) J = 6.6 1.28(d) J = 6.0	
Ile	171.5^a	56.9^b	40.1	25.2 15.3	10.9	8.70 (ca.)	5.23(m)	2.45(m)	1.80(m) 1.33(d) J = 5.8	0.94(t) J = 7.2
Thr	172.0^a	63.0	68.4	20.9		8.58 (br.s)	5.38(m)	4.45(m)	1.57(d) J = 6.0	
Pro$_1$	172.3^a	62.5	29.6	25.2	48.6^c		4.19(m)	2.06(m) 1.95(m)	1.88(m) 1.39(m)	3.83(m) 3.74(m)
Pro$_2$	172.3^a	62.0	29.6	25.2	49.0^c		3.89(m)	1.90(m) 1.69(m)	1.51(m) 1.46(m)	3.84(m) 3.73(m)
Phe$_1$	173.3^a	58.0	41.8	138.0^d	126.9 - 130.0	8.59 (br.s)	5.59(m)	3.44(d) J = 13, 4.2 3.15(m)		7.0 - 7.6
Phe$_2$	172.1^a	60.5	35.9	139.5^d	126.9 - 130.0	9.56 (br.s)	4.02(m)	4.00(m) 3.61(m)		7.0 - 7.6

a, b, c, d assignments with the same superscripts may be interchanged.

By analyzing the TOCSY, HMQC and DQF - COSY spectra, eight amino acid residues were identified as one glycine, one isoleucine, one threonine, one valine, two phenylalanine and two proline units. The molecular weight of these amino acid residues was identical with that observed in FABMS (pos.). Assignment of the ^1H and ^{13}C NMR signals (Table 2) of 4 was accomplished using combination of 2D – NMR experiments such as DQF – COSY, HMQC and TOCSY.

The sequence of these amino acid residues was determined by ROESY spectrum. The ROESY correlations (Figure 2) suggested the presence of two peptide fragments (– Pro1 – Gly – Phe1 – Val – and – Pro2 – Phe2 – Thr – Ile –), and these two peptide fragments had to be linked in only one sequence. These facts together with the information provided by FABMS led to the proposition of structure of 4 as cyclo (– Pro_1 – Gly – Phe_1 – Val – Pro_2 – Phe_2 – Thr – Ile –).

Experimental

General Melting points were determined on Kofler block and uncorrected. Optical rotations were measured with a SEPA – 300 polarimeter. IR spectra were measured on Bio – Rad FTS – 135 spectrometer. NMR spectra were obtained on Bruker AM – 400MHz, DRX – 500MHz and Varian INOVA – 600MHz spectrometers. A VG Auto Spec – 3000 spectrometer was used to record MS spectra. 200 – 300 mesh and 300 – 400 mesh silica gel (made in Qingdao Ocean Chemical Factory) were used for column chromatography and silica gel G for TLC. All solvents were industrial products, and redistilled before using.

Plant Material The dried roots of *Psammosilene tunicoides* were bought from the Yunnan Baiyao Drug Factory in Kunming, China.

Extraction and Isolation Powdered roots of *Psammosilene tunicoides* (25kg) were extracted three times with ethanol (90 %) at refluxing condition for 4 hours. Removal of solvents by evaporation yielded a syrup. The syrup was suspended in acetone, filtrated out the precipitate. The filtrate was concentrated to afford a residue. The residue was subjected to a silica gel column, eluting with $CHCl_3$ and increasing proportions of MeOH (5 – 30%), and gave four fractions. Fractions were monitored by TLC. The Fr. II (64g) were further purified by column chromatography on silica gel and RP – HPLC to gave compound 1 (17mg), compound 2 (12mg), Psammosilenin A (3) (10mg) and Psammosilenin B (4) (6mg).

Compound 1: $C_6H_{10}N_2O_2$, colorless needles (methanol), ninhydrin reaction (–). mp: 206 ~ 208℃. IRν_{max}^{KBr} (cm^{-1}): 3300, 1650. EI – MS m/z (%): 142 ($[M]^+$ 65), 114 (16), 99 (100), 84 (5), 71 (67), 56 (65). HREI – MS: $[M]^+$ at m/z 142.0733 (calcd. m/z 142.0742). ^1H NMR (400MHz, pyridine – d_5) δppm: 9.30 (2H, br. s, NH), 4.28 (2H, q, J = 8.0Hz, α – H_{Ala}), 1.63 (6H, d, J = 8.0Hz, β – H_{Ala}). ^{13}C NMR (100MHz, pyridine – d_5) δ ppm: 170.5 (CO), 51.2 (CH, α – C_{Ala}), 19.1 (CH_3, β – C_{Ala}).

Compound 2: $C_8H_{14}N_2O_2$, colorless needles (methanol), ninhydrin reaction (–). mp: 177 ~ 179℃. IRν_{max}^{KBr} (cm^{-1}): 3300, 1650. EI – MS: m/z (%) 170 $[M]^+$ (1), 128 (100), 113 (35), 99 (45), 84 (17), 72 (47), 56 (22). HREI – MS: $[M]^+$ at m/z 170.1068 (calcd. m/z 170.1055). ^1H NMR (400MHz, pyridine – d_5) δ: 9.25 (1H, br. s, NH), 8.98 (1H, br. s, NH), 4.35 (1H, m), 4.13 (1H, m), 2.67 (1H, m, β – H_{Val}), 1.66 (3H, d, J = 4.0Hz, β

$-H_{Ala}$), 1.14 (3H, d, J = 8.0Hz, $\gamma-H_{Val}$), 1.07 (3H, d, J = 8.0Hz, $\gamma-H_{Val}$). ^{13}C NMR (100MHz, pyridine $-d_5$) δ: 171.5 (CO), 167.5 (CO), 60.9 (CH), 51.2 (CH), 32.2 (CH, $\beta-C_{Val}$), 20.9 (CH_3, $\gamma-C_{Vla}$), 19.1 (CH_3, $\gamma-C_{Vla}$), 17.1 (CH_3, $\beta-C_{Ala}$).

Psammosilenin A (3): $C_{51}H_{64}N_8O_8$, white powder, ninhydrin reaction (–). $[\alpha]_D^{25}$ – 108.14 (c 0.39, MeOH). IRν_{max}^{KBr} (cm^{-1}): 3290, 1640. positive FAB – MS m/z (%): 917 $[M+H]^+$ (30), 245 (3), 217 (7), 120 (25), 70 (100). HRFAB – MS: $[M+H]^+$ at m/z 917.4859 (calcd. m/z 917.4925). ^1H NMR and ^{13}C NMR data were listed in Table 1.

Psammosilenin B (4): $C_{45}H_{62}N_8O_9$, white powder, ninhydrin reaction (–). IR ν_{max}^{KBr} (cm^{-1}): 3300, 1650. positive FAB – MS m/z (%): 859 $[M+H]^+$ (15), 415 (2), 346 (5), 302 (8), 245 (3), 155 (9), 120 (33), 70 (100). ^1H NMR and ^{13}C NMR data were listed in Table 2.

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